BlmVII. Figure 6B synthesis using NRPS, BlmVIII, and BlmVII. Figure 6C synthesis using BlmIX, BlmVIII, and BlmVII. Figure 6D synthesis using BlmIX, BlmVIII, and NRPS (C, A^N, PCP). Figure 6E synthesis using BlmIX, BlmVIII, and NRPS (C, A^C, PCP, OX). --

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Delete the paragraph at page 15, lines 18-24 and insert the following:

--Figure 8A shows a restriction map of the *blm* gene cluster from *Sv* ATCC15003 (B, *Bam*HI). 8B shows the relative position of the *blmI*, *blmII*, and *blmXI* genes to the two *blmAB* resistance genes (*blm*^{R}, Blm resistance). Individual open reading frames are represented by open arrows. Figure 8C (SEQ ID NO:127 nucleotide sequence and SEQ ID NO:128 amino acid sequence) shows the nucleotide sequence of the *blmI* gene. The potential ribosome-binding site (RBS) and the conserved motif for 4'-phosphopantetheinylation are underlined. The sequence has been deposited into GenBank under accession no. AF210249.--

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Delete the paragraph at page 15, lines 25-31 and insert the following:

--Figure 9 shows an amino acid sequence comparison of BlmI (SEQ ID NO:133) with PCP domains of known type I NRPSs (Grs-2 [P14688] (SEQ ID NO:129), 36% identity, 58% similarity; Srfa-3 [Q08787] (SEQ ID NO:130), 40% identity, 64% similarity; Vir-s [Y11547] (SEQ ID NO:131), 36% identity, 60% similarity; Saf-b [U24657] (SEQ ID NO:132), 40% identity, 54% similarity). Given in brackets are nucleotide sequence accession numbers. The shaded letters indicate similar amino acids. Consensus residues are amino acids that are similar in more than three sequences.--

Delete the paragraph at page 16, lines 1-5 and insert the following:

--Figures 11A-11D show the enzyme architecture of type I and type II PKS and NRPS. A, adenylation domain; ACP, acyl carrier protein or ACP domain; AT, acyl transferase; C, condensation protein or C domain; KS, β-ketoacyl synthase domain; KSα, β-ketoacyl synthase α subunit; KSβ, β-ketoacyl synthase β subunit; PCP, peptidyl carrier protein or PCP domain. Figure 11A illustrates a Type I PKS. Figure 11B illustrates a Type I NRPS. Figure 11C illustrates a Type II PKS. Figure 11D illustrates a Type II NRPS.--

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Delete the paragraph at page 19, lines 12-19 and insert the following:

--The nucleic acids comprising the *blm* gene cluster are identified in Tables I and II and listed in the sequence listing provided herein (SEQ ID NOS: 1 and 2, GenBank Accession numbers AF149091, AF210249, AF210311). In particular, Table I identifies genes and functions of open reading frames (ORFs) responsible for the biosynthesis of the hybrid peptide/polyketide/peptide backbone and sugar moieties of bleomycin, while Table II identifies a number of ORFs comprising the *blm* gene cluster, identifies the activity of the catalytic domain encoded by the ORF and provides primers for the amplification and isolation of that orf.--

Delete the Table 1 at pages 19-20 and insert the following:

-- **Table I.** Determined functions of ORFs in the bleomycin biosynthesis gene cluster

Gene	Amino acids	Sequence Homolog[1]	Proposed function[2, 3]
orf8	424	YqeR (BAA12461)	Oxidase
blmC	498	RfaE	NDP-glucose synthase
blmI	90	GrsB (P14688)	Type II PCP
blmD	545	NodU (Q53515	Carbamoyl transferase
blmE	390	RfaF (AAD16056)	Glycosyl transferase
orf13	187	MbtH (O05821)	Unknown
blmII	462	Nrp (CAA98937)	NRPS condensation enzyme
orf15	339	SyrP (1890776)	Regulation
blmII	935	HMWP2 (P48633), McbC (P23185)	A PCP Ox
blmIV	2626	HMWP2 (P48633)	C A PCP Cy A PCP Cy
orf18	638	AsnB (2293165)	Asparagine synthetase
blmF	494	RfbC (Q50864)/BlmOrf1 (507319)	Glycosyl transferase/β-hydroxylase
blmG	325	YtcB (2293288)	Sugar epimerase
blmV	645	McyB (2708278)	PCP C
blmVI	2675	ACoAS (1658531), PksD (S73014) SnbDE (CAA67249)	A[4] ACP C A PCP C A
blmVII	1218	SyrE (3510629)	C A PCP
blmVIII	1841	HMWP1 (CAA73127)	KS AT MT KR ACP
blmIX	1066	SafB (1171128)	C A PCP
blmX	2140	TycC (2623773)	C A PCP C A PCP
blmXI	688	SyrE (3510629)	NRPS condensation enzyme
orf28	239	SC9C7.04C (CAA22716)	Unknown
orf29	582	YvdB (CAB08068)	Transmembrane transporter

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orf30	113	SmtB (P30340)	Regulation
orf31	117	PhnA (P16680)	Unknown

--

Delete the paragraph at page 47, lines 14-21 and insert the following:

--Noteworthy are the genes encoding the putative NRPS and PKS enzymes. The *blmI, blmII,* and *blmXI* genes encode NRPSs with an unusual architecture. In contrast to all known NRPSs, which are of modular organization with each module consisting minimally of a condensation (C), an

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adenylation (A), and a peptidyl carrier protein (PCP) domain (1), BlmI, BlmII, and BlmXI are discrete proteins homologous to individual domains of type I NRPSs. We have characterized BlmI as a type II PCP. The BlmII and BlmXI proteins could serve as candidates for type II condensation enzymes. It is unclear yet what role if any these discrete NRPS enzymes could play in BLM biosynthesis.--

Delete the paragraph at page 70, line 22 - page 71, line 31 and insert the following:

--In order to test if *pptA* actually encodes a functional PPTase, we decided to overproduce and purify the PptA protein, and assay its catalytic competence on putative substrate proteins or domains. The *pptA* coding sequence was amplified by PCR and cloned into the T5-promoter-based pQE-70 vector, yielding plasmid pQEPPT, in such a way that a hexahistidine tag would be added at the C-terminus of the protein. Expression of the pQEPPT construct in *E. coli* M15(pREP4) resulted in the overproduction of soluble His-tagged PptA which was readily purified by

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affinity chromatography on Ni-NTA agarose under non-denaturing conditions. Because *pptA* belongs, by sequence similarity, to the subfamily of PPTases involved in nonribosomal peptide synthesis, we first assayed its activity using two different apo-PCPs as protein substrates. The first one, BlmI, has been previously characterized in our laboratory as a discrete peptidyl carrier protein, or type II PCP, whose gene is found within the bleomycin-biosynthesis gene cluster of *S. verticillus* (Du et al. *Chem. Biol.* (1999) 6:507-517). For the second PCP substrate we used BlmX, a bimodular NRPS protein encoded in the same cluster (Fig. 2), as a source of a type I PCP, i. e. a PCP included in a multidomain NRPS. For the production of this type I PCP, we amplified by PCR a 1,898 bp fragment encoding the adenylation and PCP domains from the second module of BlmX. This DNA fragment was cloned into pMAL-c2x to yield pMAL1617, in which the type I PCP would be produced as a maltose-binding protein (MBP) fusion, MBlmX-2, with a predicted molecular mass of 108. 5 kDa.

Introduction of pMAL1617 in *E. coli* TB1 resulted in good overproduction of MBlmX-2, about 40% soluble, which was purified by affinity chromatography using amylose resin. To test the PPTase activity, we incubated the purified PptA with BlmI and MBlmX-2 as putative protein substrates in the presence of (^{3}H)-(pantetheinyl)-CoASH, and the tritiated products were subjected to SDS electrophoresis and autoradiography. The well-characterized PPTase Sfp from *B. subtilis*, which exhibits a broad specificity for its protein substrate (Quadri et al. *Biochemistry* (1998) 37:1585-1595), was included as a positive control. In these experiments PptA exhibited a robust phosphopantetheinylation activity on both BlmI and MBlmX-2. Having demonstrated that PptA does in fact have PPTase activity on both type I and type II PCP substrates from nonribosomal peptide synthetases, we then proceeded to test two different acyl-carrier proteins (ACPs) as potential

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substrates. The first one, BlmVIII, is a monomodular multidomain polyketide synthase (PKS) which is encoded in the bleomycin-biosynthesis gene cluster of *S. verticillus* (Fig. 2). BlmVIII contains an ACP domain at its C-terminus, that is a type I ACP. For the second ACP substrate we used TcmM, a type II acyl carrier protein involved in the biosynthesis of the aromatic polyketide tetracenomycin C in *S. glaucescens* (Shen et al. *J. Bacteriol.* (1992) 174:3818-3821; Bao et al. *Biochemistry* (1998) 37: 8132-8138). For the production of TcmM, its coding sequence was transferred from a construct previously made in pET-22b (Gehring et al. *Chem. Biol.* (1997) 4:17-24) into the pET-28a vector to yield pET28a-TcmM, in such a way that a hexahistidine tag should be added at both the N-terminus and the C-terminus of the protein. Plasmid pET28a-TcmM was introduced into *E. coli* BL21(DE3), and TcmM was easily purified by affinity chromatography using Ni-NTA resin. In vitro phosphopantetheinylation assays were performed as before, but using BlmVIII and TcmM as protein substrates, and PptA was able to posttranslationally modified both ACP substrates.--

In accordance with 37 CFR §1.121 a marked up version of the above-amended paragraph(s) illustrating the changes introduced by the forgoing amendment(s) are provided in Appendix A.

In the Claims:

Please cancel claims 4, 6-8, 11, 16, 18-20, 22-39, and 46-70 without prejudice.